

L3 ANSWER 21 OF 23 CAPLUS COPYRIGHT 1999 ACS
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 TI Production of **insulin-like growth factor-1 (IGF-1)** in methylotrophic yeast cells
 IN Brierley, Russell Arthur; Davis, Geneva Ruth; Holtz, Gregory Clyde; Gleeson, Martin A.; Howard, Bradley D.
 PA Salk Institute Biotechnology/Industrial Associates, Inc., USA
 SO PCT Int. Appl., 100 pp.
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 DT Patent
 LA English
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	EP 548267	A1	19930630	EP 91-918262	19910904
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE				
	JP 06500470	T2	19940120	JP 91-516963	19910904
	US 5324639	A	19940628	US 93-23463	19930225
	US 5612198	A	19970318	US 94-308196	19940919
PRAI	US 90-578728		19900904		
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	US 93-983523		19930303		

TI Production of **insulin-like growth factor-1 (IGF-1)** in methylotrophic yeast cells
 AB Expression vectors and host cells contg. same are provided that stably express **IGF-1** and that secrete high concns. of biol. active **IGF-1**; the expression system is readily scaled up to produce large quantities of **IGF-1**. **IGF-1** is produced by growing methylotrophic yeast transformants contg. in their genome .gtoreq.1 copy of DNA encoding **IGF-1** in operational linkage with a signal sequence which includes the proteolytic processing site Lys-Arg and may include .gtoreq.1 Glu-Ala sequences. In preferred embodiments, the signal sequence is of the *Saccharomyces cerevisiae* .alpha.-mating factor pre-pro sequence. Expression of both the DNA encoding **IGF-1** and the pre-pro signal sequence are regulated by a promoter region derived from a methanol-responsive gene of a methylotropic yeast. DNA constructs and recombinant methylotrophic yeast strains used for the expression and secretion of **IGF-1** are also provided. For preferred embodiments, protease-deficient *Pichia pastoris* strains are provided. Ferms. and characterization of the **IGF-1** secreted into the culture medium (including amino acid compn. and sequence) are described. High-level prodn. of **IGF-1** by *P. pastoris* was readily scaled up to a 10-L fermn. level.
 ST **insulin like growth factor 1** yeast; somatomedin C prodn methylotrophic yeast; *Pichia somatomedin C* recombinant prodn
 IT Plasmid and Episome
 (DNA for **insulin-like growth factor 1** on, for **insulin-like growth factor 1** prodn. in methylotrophic yeast)
 IT Deoxyribonucleic acid sequences
 (for **insulin-like growth factor 1** DNA of human)

IT Protein sequences
 (for **insulin-like growth factor**
 1 of human)

IT Deoxyribonucleic acids
 RL: BIOL (Biological study)
 (for **insulin-like growth factor**
 1 prodn. in methylotrophic yeast)

IT *Pichia pastoris*
 (**insulin-like growth factor 1**
 recombinant prodn. in)

IT Fermentation
 (of **insulin-like growth factor**
 1, in *Pichia pastoris*)

IT Plasmid and Episome
 (pIGF204, DNA for **insulin-like growth**
 factor 1 on, for **insulin-like**
 growth factor 1 prodn. in methylotrophic yeast)

IT Plasmid and Episome
 (pIGF206, DNA for **insulin-like growth**
 factor 1 on, for **insulin-like**
 growth factor 1 prodn. in methylotrophic yeast)

IT Plasmid and Episome
 (pIGF816, DNA for **insulin-like growth**
 factor 1 on, for **insulin-like**
 growth factor 1 prodn. in methylotrophic yeast)

IT *Saccharomyces cerevisiae*
 (.alpha.-mating factor pre-pro sequence from, for signal sequence for
 insulin-like growth factor 1
 prodn. in methylotrophic yeast)

IT Gene, microbial
 RL: BIOL (Biological study)
 (AOX1, methanol-responsive gene of methylotrophic yeast and
 transcription terminator derived from, of *Pichia pastoris*,
 for **insulin-like growth factor**
 1 recombinant prodn.)

IT Yeast
 (methylotrophic, **insulin-like growth**
 factor 1 recombinant prodn. in)

IT Plasmid and Episome
 (pIGF201, DNA for **insulin-like growth**
 factor 1 on, for **insulin-like**
 growth factor 1 prodn. in methylotrophic yeast)

IT Plasmid and Episome
 (pIGF202, DNA for **insulin-like growth**
 factor 1 on, for **insulin-like**
 growth factor 1 prodn. in methylotrophic yeast)

IT Genetic element
 RL: BIOL (Biological study)
 (promoter, from methanol-responsive gene of methylotrophic yeast, for
 insulin-like growth factor 1
 recombinant prodn.)

IT Microbial hormones and pheromones
 RL: BIOL (Biological study)
 (.alpha.-factor, of *Saccharomyces cerevisiae*, pre-pro sequence from,
 for signal sequence for **insulin-like growth**
 factor 1 prodn. in methylotrophic yeast)

IT 67-56-1, Methanol, uses
 RL: USES (Uses)
 (-responsive gene, promoter from, of methylotrophic yeast, for
 insulin-like growth factor 1
 recombinant prodn.)

IT 29586-66-1 141098-54-6 141098-55-7 141170-32-3
 RL: PRP (Properties)
 (DNA encoding processing sequence of, in **insulin-like**
 growth factor 1 recombinant prodn. in methylotrophic

yeast)
 IT 66795-41-3, **Insulin-like growth factor I** (human reduced) 97199-09-2
 RL: BIOL (Biological study)
 (amino acid sequence of and recombinant expression of DNA for, complete)
 IT 144132-72-9, Deoxyribonucleic acid (human clone pIGF101 **insulin-like growth factor I**-specifying)
 RL: BIOL (Biological study)
 (nucleotide sequence and expression in *Pichia pastoris* of, complete)
 IT 144132-71-8, Deoxyribonucleic acid (human clone pIGF101 **insulin-like growth factor I**-specifying plus 5'- and 3'-flanking region fragment)
 RL: BIOL (Biological study)
 (nucleotide sequence of and expression in *Pichia pastoris* of)
 IT 67763-96-6P, **Insulin-like growth factor 1**
 RL: PREP (Preparation)
 (prodn. of, in methylotrophic yeast)
 IT 9001-92-7, Protease 9046-67-7, Carboxypeptidase Y 37228-80-1
 37288-81-6
 RL: BIOL (Biological study)
 (*Pichia pastoris* deficient for, in **insulin-like growth factor 1** recombinant prodn.)

L3 ANSWER 22 OF 23 TOXLIT
 AN 1993:32458 TOXLIT
 DN CA-118-095582H
 TI Genes influencing proteolytic activity in *Pichia*, and protein manufacture with protease-deficient *Pichia*.
 AU Gleeson MA; Howard BD
 SO (1992). PCT Int. Appl. PATENT NO. 92 17595 10/15/92 (Salk Institute Biotechnology).
 CY United States
 DT Patent
 FS CA
 LA English
 OS CA 118:95582
 EM 199305
 AB Genes involved with proteolysis in *Pichia* are cloned, and *Pichia* strains with these genes inactivated are provided for manuf. of proteinase-sensitive proteins. The genes for proteinase A (PEP4) and proteinase B (PRB-1) and the orotidine-5'-phosphate decarboxylase gene (URA3) of *P. pastoris* were cloned and sequenced. PEP4-URA3- *P. pastoris* Strains were prepd. and a gene for **insulin-like growth factor 1** (IGF1) introduced. These strains produced more IGF1 than did strains contg. the wild-type PEP4 gene.

L3 ANSWER 23 OF 23 TOXLIT
 AN 1993:32002 TOXLIT
 DN CA-118-095126N
 TI purification of intact, correctly-folded **insulin-like growth factor-1**.
 AU Holtz GC; Brierley RA
 SO (1992). PCT Int. Appl. PATENT NO. 92 12993 08/06/92 (Salk Institute Biotechnology/Industrial Associates, Inc.).
 CY United States
 DT Patent
 FS CA
 LA English
 OS CA 118:95126
 EM 199305
 TI purification of intact, correctly-folded **insulin-like**

growth factor-1.
AB Intact, correctly-folded, monomeric **insulin-like growth factor-1 (IGF-1)** is recovered and purified by 2 series of chromatog. on cation exchange exchange and hydrophobic interaction adsorbents ending with gel filtration chromatog. Human **IGF-1** was purified from the fermn. broth of recombinant *Pichia pastoris* by cation exchange chromatog. on Sp-250 ZetaPrep or Toyopearl SP550C, hydrophobic interaction chromatog. on TosoHaas Bu Toyopearl-650M, cation exchange chromatog. on Fast Flow S-Sepharose, a
2nd chromatog. on the 650M resin, and gel filtration chromatog. on Superdex
75 or Toyopearl HW50F.